marine particulate feeders, p. 77–91. *In* J. R. Nixon [ed.], Microencapsulation. Dekker.

———, A. KANAZAWA, AND U. ONO. 1979. Studies on the nutritional requirements of the larval stages of *Penaeus japonicus* using microencapsulation diets. Mar. Biol. **54:** 261–267.

LANGDON, C. J., AND M. J. WALDOCK. 1981. The effect of algal and artificial dicts on the growth and fatty acid composition of *Crassostrea gigas* spat. J. Mar. Biol. Assoc. U.K. **61:** 431–448.

LEVINE, D. M., S. D. SULKIN, AND L. VAN HEVKELUM. 1983. The design and development of microencapsulated diets for the study of nutritional requirements of brachyuran crab larvae, p. 193–203. *In* C. J. Berg, Jr. [ed.], The culture of marine invertebrates: Selected readings. Hutchinson Ross.

LOPEZ, M. D. G., M. E. HUNTLEY, AND P. F. SYKES. 1988. Pigment destruction by *Calanus pacificus*: Impact on the estimation of water column fluxes. J. Plankton Res. **10:** 715–734.

PENRY, D. L., AND B. W. FROST. 1990. Re-evaluation of the gut-fullness (gut fluorescence) method for inferring ingestion rates of suspension-feeding copepods. Limnol. Oceanogr. **35:** 1207–1214.

RAI, H. 1973. Methods involving the determination of photosynthetic pigments using spectrophotometry. Int. Ver. Theor. Angew. Limnol. Verh. **18:** 1964–1975.

RIEMANN, B. 1976. Studies on the biomass of phytoplankton. Ph.D. thesis, Rep. Bot. Inst. Univ. Aarhus, No. 1. 186 p.

SAND-JENSEN, K. 1976. A comparison of chlorophyll *a* determinations of unstored and stored phytoplankton filters extracted by methanol and acetone. Vatten **4:** 337–341.

SHUMAN, F. R., AND C. J. LORENZEN. 1975. Quantitative degradation of chlorophyll by a marine herbivore. Limnol. Oceanogr. **20:** 580–586.

SOMMERVILLE, G. R. 1962. U.S. Patent 3,015,128.

Submitted: 30 November 1990
Accepted: 2 October 1991
Revised: 11 November 1991

Limnol. Oceanogr., 37(2), 1992, 413–424

Lipids and life-cycle strategy of a hypolimnetic copepod in Lake Michigan

Abstract—Concentration and composition of lipids varied seasonally in adult female *Diaptomus sicilis*, a hypolimnetic suspension-feeding calanoid copepod, in Lake Michigan. Triacylglycerol (TG) was the predominant storage lipid; its level remained high (33–40% of dry wt) from June through January. During the period of reproduction from February through May, TG dropped steeply to a low of 9%. The abrupt increase in TG concentration from May to June was probably caused by the recruitment of nonreproducing adult females. Throughout summer and fall ovaries remained unripe. Feeding experiments at satiating food concentrations at 6° and 20°C suggest that low temperature and the hypolimnion was a major reason that ovaries did not ripen. Stored TG served as a potential buffer to starvation, although starvation conditions did not occur. By remaining in the hypolimnion rather than in the epilimnion, *D. sicilis* is assured a relatively stable supply of algae, low metabolic rate, and escape from predation. These advantages may have to be weighed against a reproductive bottleneck of low temperature.

Lipids are important energy reserves in freshwater and marine zooplankton. A vast literature exists on lipids in marine zooplankton, but relatively little work has been done on freshwater zooplankton, particularly calanoid copepods (Arts and Sprules 1987; Cavaletto et al. 1989), and work on seasonal lipid patterns has been limited to a few studies (Siefken and Armitage 1968; Hairston 1979) based on indirect measurement (i.e. dry-weight changes following chloroform-methanol extraction of lipids). A major question that arises from studies of marine copepods is: does seasonal variability in lipid stores result from changes in food availability or from reproductive condition? The roles of lipids for survival during periods of low food abundance and for

Acknowledgments

We thank G. Laird Pernie for providing unpublished temperatures and chlorophyll data; A. S. Brooks for arranging ship time on the RV *Neeskay* for collection of a critical winter zooplankton sample; L. R. Herche for statistical advice; and M. T. Arts, M. S. Evans, M. D. Ohman, C. E. Williamson, and an anonymous reviewer for critical comments on the manuscript.

Contribution 744 from the GLERL.

reproduction are recognized for freshwater Cladocera (Tessier et al. 1983), but not for freshwater calanoids.

Previously we described the seasonal feeding biology of adult female *Diaptomus sicilis,* a hypolimnetic suspension-feeding (sensu Vanderploeg 1990) calanoid copepod found at all times of year (Vanderploeg et al. 1984). In summary, *D. sicilis* females found modest resources throughout the year, with somewhat lesser food concentrations in late summer and fall. The reproductive peak of *D. sicilis* is in late winter and spring. We were curious why reproduction did not occur at other times and whether *D. sicilis* may be stressed in late summer—the time of low food concentration and possible competition for it from cladocerans. By addressing these issues we also hoped to answer the question of whether lipid dynamics are driven by food supply or reproduction. Insight into these questions about the life-cycle strategy of *D. sicilis* was obtained by monitoring seasonal food concentrations, lipids, and reproductive status and examining temperature effects. Lipid levels represent an integration of food input and losses due to respiration and reproduction.

Diaptomus sicilis was collected approximately monthly from January 1986 to April 1987 in vertical tows of a 57-cm-diameter net with 153-μm mesh at either an 80- or 100-m-deep station located, respectively, 15 and 21 km west of Grand Haven, Michigan. It is difficult to sample Lake Michigan from Grand Haven and other ports on the eastern shore in winter because prevailing winds pile up ice in the nearshore region. Thus collections were also made in winter 1986 on Grand Traverse Bay, a 190-m-deep bay on northern Lake Michigan, and at a 100-m-deep station near Milwaukee, Wisconsin, for the February 1987 sample.

Adult *D. sicilis*' center of distribution is probably the upper hypolimnion (Bowers 1980; Vanderploeg et al. 1984). Therefore, during the stratified season water collected for triplicate or quadruplicate measurements (SE/$\bar{x}$ < 5%) of fluormetrically determined chlorophyll (corrected for pheophytin) (Strickland and Parsons 1972) was taken from the upper hypolimnion to give an estimate of food availability. For comparison of the feeding conditions in the hypolimnion and epilimnion, water collected between 4 and 10 m was used to estimate the chlorophyll concentration of the epilimnion. During isothermal seasons water was collected at a depth between 2 and 10 m (shallow) and sometimes at 50 m (deep) to verify that the shallow measurements characterized the full water column. An exception to this procedure was that the entire water column was sampled for chlorophyll for the March 1986 sampling of Grand Traverse Bay, which was covered with ice at the time. Because of the high transparency of the ice and the water-column stability provided by ice cover, a broadly distributed bloom of algae developed between 2 and 60 m, bracketing the depth range of *D. sicilis* (Vanderploeg et al. in press). The 4-m chlorophyll value reported here is characteristic of the bloom over its broad depth range. Temperatures were determined by an electronic bathythermograph.

Contents of net tows were placed in an insulated 11-liter picnic jug filled with lake water from the upper hypolimnion. About 300 adult females were sorted the day after sample collection for the analyses described below. During this 1-d interval there should have been little loss of lipid due to starvation at ambient lake temperatures (*see below*).

Fifty adult females were placed in each of five small test tubes (6-mm diam × 50 mm long) to obtain sufficient mass and replicates for precise determination of dry weights and lipid composition. The replicate samples were dried in a desiccator at 50°C for 2 d under a slow, steady flow of nitrogen. The dried samples were kept frozen under vacuum in a desiccator that had been purged with nitrogen to prevent lipid oxidation. Total lipid was extracted and quantified gravimetrically by a microadaptation (Gardner et al. 1985) of the Folch et al. (1957) procedure. A subsample of lipid extract was saved for determination of lipid classes by thin-layer chromatography with flame-ionization detection (Parrish 1987). The lipid extract was spotted directly onto silica-coated Chromarods-SII (Ancal Inc.) and sequentially scanned with a Iatroscan Mark IV (Iatron Labs.) connected to a Hew-

lett-Packard 3392A integrator. The lipid classes were separated by developing the rods in an increasing-polarity solvent system of hexane/diethyl-ether/formic acid (99 : 1 : 0.05), hexane/diethyl-ether/formic acid (80 : 20 : 0.1), 100% acetone, and dichloromethane/methanol/water (5 : 4 : 1) (Parrish 1987). In a comparison between the Iatroscan and the gravimetric method for total lipid determination in *D. sicilis*, the sum of Iatroscan-measured lipid classes was 87±18% ($\bar{x}$ ±1 SD, n = 14) of the gravimetrically determined value.

Twenty-five animals, freshly narcotized with club soda, were visually examined at each sampling. Body volumes (V) were determined by assuming an ellipsoid metosome and a urosome having the cross section of an ellipse from measurements of prosome length (a), width (b), and depth (c), and urosome width (d), depth (f), and length (l) with the formula $V = \pi[(abc)/6 + (dfl)/4]$ (Vanderploeg et al. 1984). Lipid content was quantified by a lipid index (Fig. 1). Reproductive condition was assessed by determining whether the females were carrying eggs and by an ovary index that quantified the development of the ovaries (Fig. 1). This index, having a scale ranging between 0 and 3, was designed to correspond to the stages of ovary development described by Marshall and Orr (1952): 1—medium (stage II); 2—semiripe (III); 3—ripe (IV); and 0—early or immature (I) or spent (V), because we could not distinguish between early and spent females. The lipid index also ranged between 0 and 3 (Fig. 1). From these same data, we also calculated the *f* index of Williamson and Butler (1987)—the percentage of females neither carrying eggs nor having visible ovaries. They claimed that a high value of this index indicated food limitation.

To determine the ability of adult female *D. sicilis* to survive without feeding during winter and summer, we placed them in 0.2-μm-filtered lake water at ambient temperature in dim light (~8 μmol quanta m^{-2} s^{-1} from "cool-white" fluorescent tubes) on a light/dark cycle to match the ambient cycle. The winter experiment (started 4 February 1987) was run with 326 animals in one 2-liter beaker at 2°C. The summer experiment

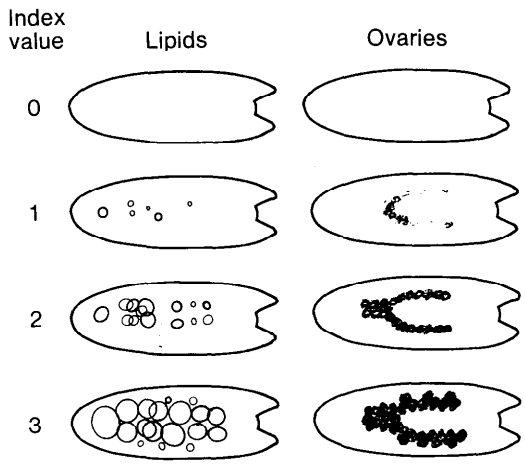

Fig. 1. Appearance of ovary development and lipid droplets for different ovary and lipid index values, patterned after Marshall and Orr (1952) and Tessier and Goulden (1982).

(started 31 August 1988) was run with 100 animals in one 1-liter beaker at 6°C. Beakers were examined weekly to remove dead animals and replace the lake water with freshly filtered lake water.

We had observed that during summer (*see below*), lipid contents were quite high, yet ovaries were not well developed. We suspected that low temperature limited ovary development rate because temperature and not photoperiod regulates reproductive rate in other species of *Diaptomus* (Watras 1983; Williamson and Butler 1987); therefore, we did the following experiment. On 17 September 1986, the usual lipid analyses were done on ~300 freshly captured females. On 19 September 1986, 300 more animals from this same collection were placed in a 2.5-liter bottle filled with a 3 mm^3 $liter^{-1}$ suspension of a 1 : 1 (cell vol. : cell vol.) mixture of *Chlamydomonas* sp. (UTEX 796) and *Cryptomonas reflexa* (from C. E. Williamson) in 0.22-μm-filtered Lake Michigan water.

Algae were grown in unbuffered WC medium (Guillard and Lorenzen 1972) at 15°C at a light intensity of 70 μmol quanta m^{-2} s^{-1} on a 16 : 8 L/D cycle. Ingestion rate saturates at a concentration of ~0.8 mm^3 $liter^{-1}$ (Vanderploeg et al. 1984). The bottle was placed on a rotating wheel at 0.5 rpm in an incubator in dim light with a 12 : 12

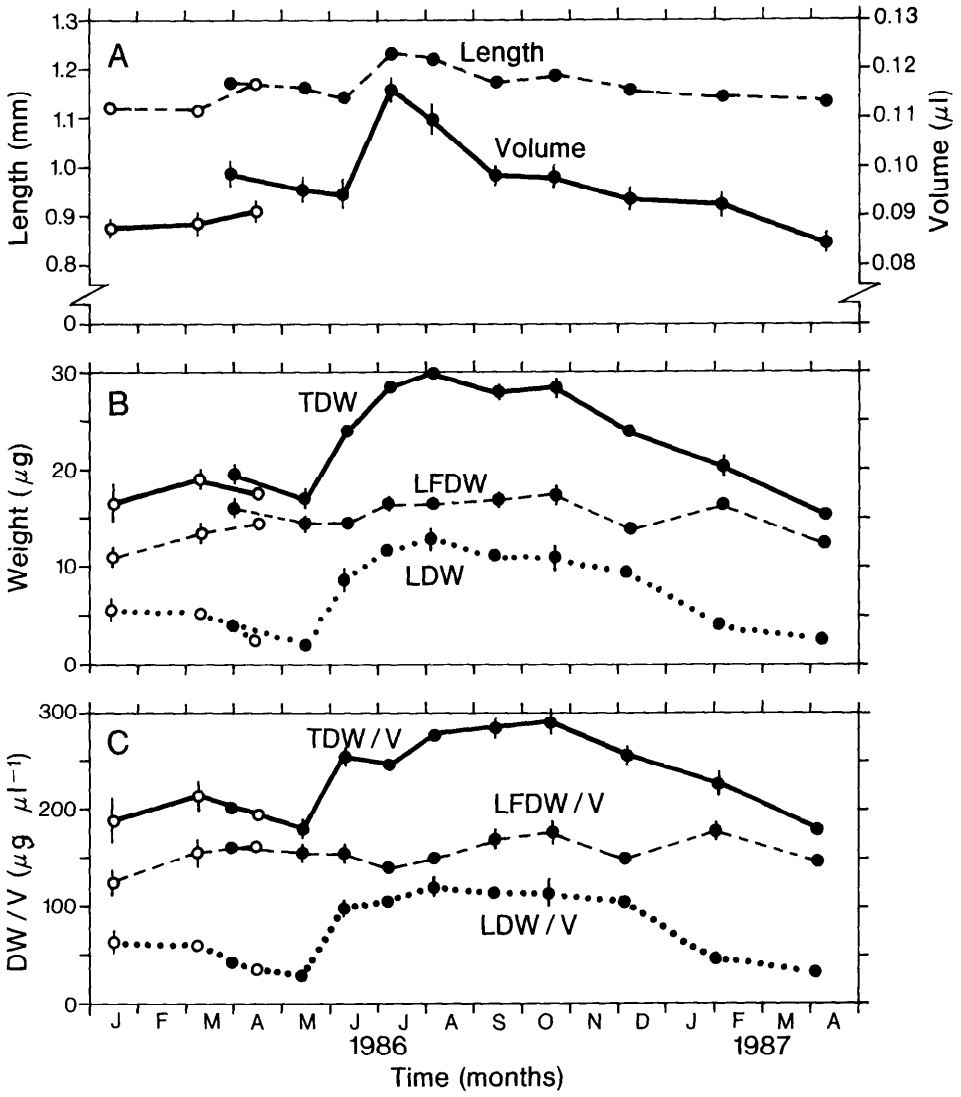

Fig. 2. Seasonal values (±1 SE) of length and volume (n = 25); weight (n = 5) expressed as total dry weight (TDW), lipid dry weight (LDW), and lipid-free dry weight (LFDW); and TDW (n = 5) per unit of volume (TDW/V), LDW per unit of volume (LDW/V), and LFDW per unit of volume (LFDW/V). Where error bars are not present, errors were too small to be drawn on figure. Data from Grand Traverse Bay—○; from open Lake Michigan—●.

L/D cycle at 6°—the temperature of the upper hypolimnion. Temperature varied between 6° and 10°C during the first 5 d because of problems with the incubator. Fresh algal suspension was added twice a week to maintain algal concentration and animals were transferred weekly to a new suspension of algae in freshly filtered lakewater.

Twice a week, 20 live animals were examined (without harm) in a small volume of water for lipid and ovary indices. On 29 September 1986, 30 animals were taken from the 6°C treatment and placed in a 20°C incubator in a 300-ml bottle at the same food concentration and light conditions. They were also monitored for lipid and ovary indices as described above, except that 15 individuals were examined each time. About 1 month later, ~300 animals in the 6°C treatment were quantitatively analyzed for lipid content and composition.

With minor modifications, this experi-

Table 1. Seasonal variation of major lipid classes in *Diaptomus sicilis*. Upper values are percent of total lipids [$\bar{x} \pm 1$ SE (n)]; lower values are concentrations per unit of body volume ($\mu g\ \mu l^{-1}$). Other classes (with $\bar{x} \pm 1$ SE of all dates for percent of total lipid indicated in parentheses) included: hydrocarbons (0.44±0.16), wax and sterol esters (0.54±0.16), methyl esters (0.24±0.12), alcohols (0.14±0.10), diglycerides (0.29±0.11), and non-lipid material (0.73±0.28).

	Triacylglycerides	Phospholipids	Acetone mobile polar lipids*	Free fatty acids	Sterols
13 May 86	60.9±3.2(4)	20.3±3.9(4)	5.62±1.32(4)	3.65±0.59(4)	3.54±0.68(4)
	15.9	5.31	1.46	0.954	0.902
9 Jun 86	83.2±2.3(4)	5.38±0.84(4)	4.50±0.47(4)	2.50±0.70(4)	1.69±0.32(4)
	82.9	5.36	4.48	2.49	1.68
7 Jul 86	87.5±2.3(3)	5.14±0.77(3)	2.36±0.45(3)	3.09±1.56(3)	1.34±0.36(3)
	92.1	5.40	2.48	2.90	1.41
4 Aug 86	89.6±2.1(4)	4.62±0.92(4)	1.74±0.28(4)	1.11±0.22(4)	2.43±0.90(4)
	107.9	5.57	2.09	1.34	2.93
16 Sep 86	84.2±2.1(4)	4.29±0.94(4)	5.48±0.64(4)	1.92±0.46(4)	1.90±0.49(4)
	96.1	4.87	6.22	2.18	2.16
20 Oct 86	79.7±2.0(3)	8.80±0.50(3)	3.83±1.13(3)	1.67±0.95(3)	4.07±1.89(3)
	89.7	9.90	4.30	1.88	4.58
8 Dec 86	86.8±1.1(4)	6.33±0.62(4)	1.60±0.56(4)	2.58±0.43(4)	0.98±0.51(4)
	90.1	6.57	1.66	2.68	1.02
2 Feb 87	69.3±2.7(4)	12.90±3.95(4)	11.20±5.32(4)	2.58±1.26(4)	2.98±0.98(4)
	32.2	6.00	5.21	1.20	1.38
7 Apr 87	62.4±3.7(5)	17.12±2.24(5)	7.18±0.43(5)	5.54±0.67(5)	3.86±0.34(5)
	19.8	5.45	2.29	1.77	1.23
$\bar{x}$±SD	78.2±11.1	9.43±5.96	4.83±3.05	2.74±1.29	2.52±1.12
	69.6±36.1	6.05±1.52	3.35±1.72	1.93±0.68	1.92±1.18

* Includes glycolipids, monoglycerides, and pigments.

ment was repeated a year later. This time 100 animals were placed in 2.5-liter bottles for both the 6° and 20°C treatments and the algal concentration was 2 rather than 3 mm^3 $liter^{-1}$. Also, 25 rather than 15 or 20 animals were examined for lipid and ovary indices.

Diaptomus sicilis exhibited small changes in length and modest changes in volume with season (Fig. 2A). Since lipid-free dry weight (LFDW) was stable and lipid weight (LDW) was variable, lipid content was a major source of variance in total dry weight (TDW) of the animals (Fig. 2B). The same pattern emerged in LFDW and LDW per unit of volume (LDW/V and LFDW/V in Fig. 2C). First-order error analysis (e.g. Bevington 1969) for TDW, where TDW = V(LFDW/V + LDW/V), indicated the following relative variance contributions: 67% from LDW/V, 22% from V, and 10% from LFDW/V. Thus variation in lipid concentration was the major source of dry-weight variation in *D. sicilis*.

Total lipid concentrations were high from June through December, decreased in winter and spring, and were lowest in April and May (Figs. 2 and 3C). The high lipid levels during summer through early winter (39–44% of dry wt) are much higher than those reported for freshwater Cladocera (Tessier et al. 1983) and are comparable to maxima for *Diaptomus minutus* in Canadian lakes (Arts and Sprules 1987) and for three species of *Diaptomus* from ponds in Kansas (Siefken and Armitage 1968).

Maximal lipid levels in *D. sicilis* are comparable to those of midlatitude marine calanoid copepods but are considerably lower than those reported for high-latitude copepods, which in the case of *Calanus hyperboreus* had a lipid level that exceeded 70% of dry mass (Bamstedt 1986). Triacylglycerol (TG), the usual storage lipid of most freshwater zooplankton (Cavaletto et al. 1989), was the dominant lipid component in *D. sicilis* and accounted for 61–90% of lipid content by weight (Table 1). Both wax ester and free alcohol constituted <1% of total lipids (Table 1). Free alcohols are esterified with fatty acids to make wax esters—a major storage product in high-latitude marine copepods and the freshwater copepods *Limnocalanus macrurus* and *Senecella calanoides* (Cavaletto et al. 1989).

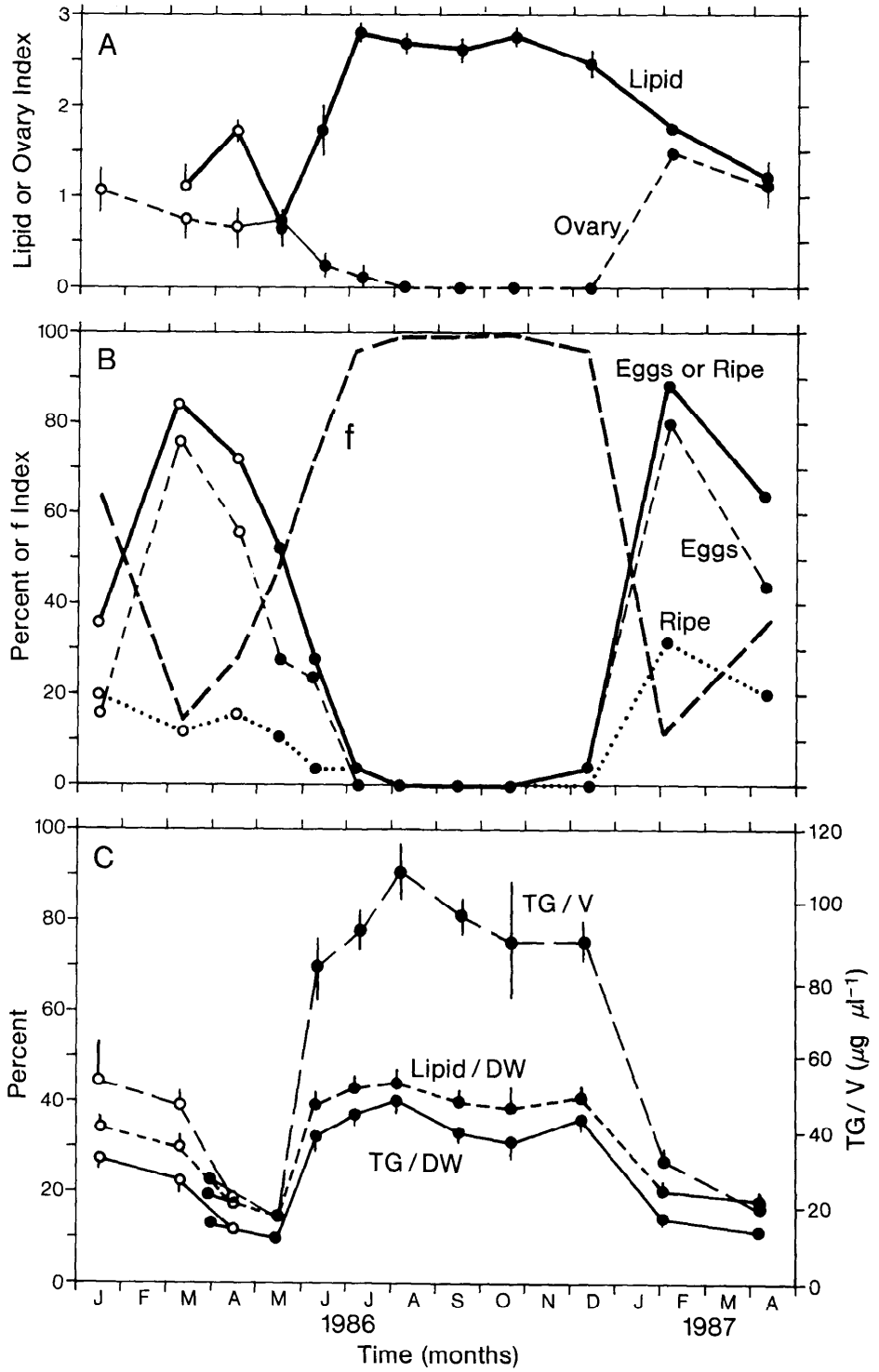

Fig. 3. Seasonal values (± 1 SE) of lipids, reproductive condition, and *f* (food limitation) index (Williamson and Butler 1987) in female *Diaptomus sicilis*. Data from Grand Traverse Bay—○; from open Lake Michigan—●. A. Lipid and ovary indices (n = 25). B. *f* index and percentage of females carrying eggs, having ripe ovaries (ovary index = 3), or being in either condition (n = 25). C. Lipid concentration (n = 5) expressed as lipids as percent of dry weight (DW), triacylglycerides (TG) as percent of DW, and TG per unit of volume (TG/V).

The second most important lipid component, phospholipids, accounted for between 5 and 20% of total lipid composition. Consistent with the role of phospholipids as an essential structural and functional component of all membranes and organelles, phospholipid concentration ($\mu g\ \mu l^{-1}$) was relatively stable with season (Table 1). Seasonally, TG plus phospholipids ranged from 80 to 94% of total lipid composition (Table 1).

Because of the constancy in levels of the secondary lipid component, the phospholipids, and dominance of TG, TG levels (expressed as percent of LDW) were strongly correlated ($r^2 = 0.96$) with total lipid levels (expressed as percent of total dry wt): $y = 48.6 + 0.899X$, $P < 0.01$. We used this regression to predict TG levels from total lipid levels for January through April 1986, when we had no data on composition (Fig. 3C). As expected, the seasonal pattern for TG is very similar but levels are lower than total lipid levels (Fig. 3C). Because TG is the major storage product, used both for survival during periods of low food abundance and for energy stores passed on to the eggs, further discussion of lipids will focus on triacylglycerol.

The seasonal pattern of the lipid index (Fig. 3A) exhibited the same general trends as TG level and concentration (Fig. 3C). Also, the lipid index showed reasonably good correlation ($r^2 = 0.68$, $P < 0.01$) with TG concentration expressed as weight per unit of volume ($\mu g\ \mu l^{-1}$): $y = 0.95 + 0.17X$, $P < 0.01$. Some of the unexplained variance ($1 - r^2 = 1 - 0.68 = 0.32$) must come from TG in developing ova and eggs carried in sacs because our lipid index would not include this TG. The other part must come from the limitation in an index requiring quantification of visual impression. We evaluated the index because lipid analyses are time consuming and require many individuals. Our results suggest that the index is a useful approximate quantitative tool. We used it to monitor TG changes in small numbers of animals used in the experiments to evaluate the effect of temperature on reproduction.

By dwelling in the hypolimnion, *D. sicilis* experiences low temperatures (1°–7°C) throughout the year (Fig. 4A). During much of the stratified period (starting in mid-May) and fall turnover (early December), *D. sicilis* experiences temperatures of ~4°–6°C. Water cools through fall and winter, reaching its lowest temperature, ~1°C, in March. Thus, *D. sicilis* experiences temperatures typically associated with high-latitude surface water or great depths of the oceans.

Chlorophyll concentrations were relatively high during spring through midsummer with midsummer values in the epilimnion being about the same or much lower than in the hypolimnion, where *D. sicilis* resides (Fig. 4B). These high values from the upper hypolimnion represent a part of the deep chlorophyll layer that has a broad depth distribution (e.g. Torke 1975). Lowest chlorophyll concentrations are seen from late summer through early winter.

By dwelling in the hypolimnion rather than epilimnion, *D. sicilis* often experiences higher food concentrations, and the low hypolimnetic temperatures lead to low respiratory demand (e.g. Comita 1968). *D. sicilis* feeds actively throughout the year (Bowers 1980; Vanderploeg et al. 1984, in press) and we have not seen inactive animals suggestive of a diapause phase. Clearance and feeding-rate data of Bowers (1980) and analyses presented by Vanderploeg et al. (1984, in press) suggest that feeding rate is proportional to algal (chlorophyll) concentration and independent of temperature (1°–20°C). This conclusion follows because low-quality greens and blue-greens are not dominant components of the algae, and the effective (selectivity weighted) food concentration : total food concentration ratio (a measure of food availability) is relatively constant throughout the year (Vanderploeg et al. 1984).

On average, the seston concentration found in the upper hypolimnion provides *D. sicilis* an ingestion rate equivalent to 7% of total body C per day, a value equivalent to a third of the maximal ingestion rate (Vanderploeg et al. 1984). Therefore, we may characterize the food supply as modest and relatively stable; although chlorophyll concentrations show peaks and troughs, algal food quantity and quality do not show the extreme variability seen in eutrophic lakes

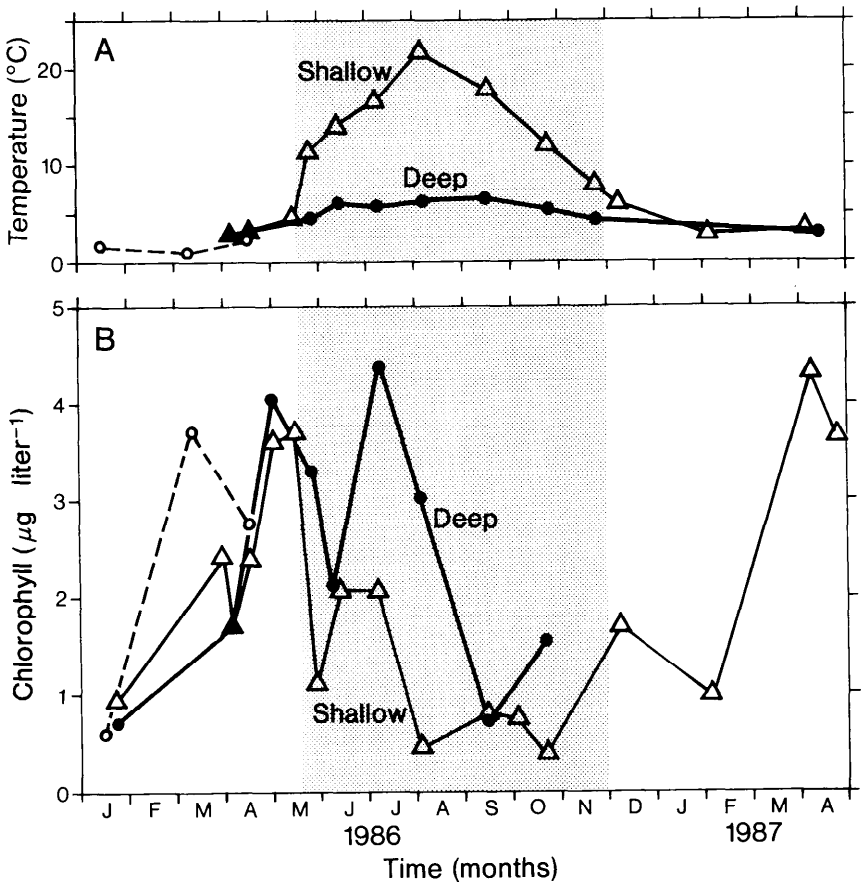

Fig. 4. Temperature and chlorophyll concentrations of shallow and deep water. Circles are values from 4-m depth of Grand Traverse Bay; other symbols are for open Lake Michigan. During the thermally stratified period (indicated by stippling), shallow water (4–10 m) is from the epilimnion and deep water is from the upper third of the hypolimnion. During isothermal seasons (when uniform depth distribution of chlorophyll is expected), deep water is from 50 m and shallow water from between 2 and 10 m.

such as Lakes Erie and Ontario (Vanderploeg 1990). Thus, the balance between feeding rate and respiration does not seem to be the primary factor affecting the dramatic changes in TG concentration (Fig. 3C). Note that during March through May, when feeding rates should be highest and respiration (assumed to be temperature driven) near its lowest point, TG concentrations are lowest. Feeding-rate experiments conducted on natural seston on the collection dates in January, March, and April 1986 verified that feeding rates in March and April were indeed high, as expected from the high chlorophyll concentrations at this time (Vanderploeg et al. in press).

Egg production appeared to be the major factor determining lipid concentration in *D. sicilis*. Whether egg production was quantified by the ovary index, proportion of females with ripe ovaries, or proportion of females carrying eggs in an external sac, TG concentration dropped through the reproductive period (January–May) and then sprang up in June with the appearance of "new" females with immature ovaries and high *f*-index values (Fig. 3B). This combination of high TG concentration and ovaries with an ovary index of 0 strongly implies these individuals were newly recruited (molted) stage VI females. Their ovaries remained undeveloped throughout summer,

fall, and early winter. At the same time lipid concentration decreased slowly.

Our data complement the limited knowledge on the population dynamics of *D. sicilis* in Lake Michigan (Torke 1975). Populations of adult female and male copepods reached their lowest numbers at the end of the major reproductive season during January through June 1973. On average the sex ratio was 1 : 1. The adult female population increased slowly throughout summer and fall, reaching a maximum in November. In Torke's study, a small pulse of eggs occurring in early October was thought to contribute to the maximum in adult population size seen in mid-November. In our study no egg production occurred until December, and then it was small. Torke described the adults, presumably arising from October egg production, as the second generation and thought that this second generation "overwintered" and was responsible for the major egg production from January through June 1974. Our lipid and reproductive data suggest that *D. sicilis* females were recruited throughout summer and fall 1986 and that survivors reproduced in winter and spring. The extended reproductive period should have led to recruitment over an extended period. Our interpretation of life history would be simpler if we knew more about population dynamics and lipid concentrations in naupliar and copepodite stages of *D. sicilis*. This research has not been done because four species of *Diaptomus* exist in Lake Michigan (Torke 1975). The different developmental stages of the various species have not been identified in an ecological study, although it is possible (but tedious) to do so.

The TG data (Fig. 3C) and reproductive parameters (Fig. 3A,B) suggest that the newly recruited females have high lipid concentrations initially or obtain them very quickly. The modest drop in TG during August through November may reflect lower food availability (Fig. 4B).

Published respiration data on *Diaptomus* spp. (Comita 1968) and the large lipid stores of *D. sicilis* in late summer suggest that *D. sicilis* could survive starvation on its lipid stores for a long time at the low temperatures of the hypolimnion. Note that it would not be expected to live very long (~10 d) at typical (~20°C) epilimnetic temperatures. Starvation experiments conducted during winter–spring, the reproductive season, and during late summer showed that *D. sicilis* can indeed survive long periods without food (Table 2; Fig. 5) at ambient temperatures. The longer survival time for the animals at 6°C than at 2°C is probably caused by the higher lipid reserves of these nonreproducing females (Table 2) since respiration is expected to be lower at 2°C. It is surprising that the winter–spring animals survived as long as they did considering that their TG supply was predicted to last for only 13 d (Table 2). Perhaps these animals lowered their metabolic rates to compensate for starvation.

The preliminary ovary-maturation experiments with satiating algal concentrations during late summer, when the animals had high lipids, showed that increasing the temperature from 6° to 20°C resulted in rapid maturation of the ovaries (Fig. 6). This result and the lack of (Fig. 6A) or much slowed (Fig. 6B) maturation of the ovaries at 6°C suggest that low temperature is a bottleneck to ovarian development; that is, ovaries develop slowly at the temperatures that *D. sicilis* experiences throughout the year. The fact that the ovaries did start to mature in the laboratory at 6°C (Fig. 6B) but not in the field suggests a secondary effect of food concentration.

In the laboratory experiments, lipid concentrations did not increase despite satiating food concentrations. In the case of the treatments at 20°C, a drop in the lipid index corresponded to increases in ovary development (Fig. 6). No such drop was seen in the lipid index for the 6°C treatments. TG/V at the end of the 6°C treatment shown in Fig. 6A [75.8 ± 1.7 ($n = 2$) $\mu g\ \mu l^{-1}$] was slightly, but not significantly, lower than in animals at the beginning of the experiment [96.9 ± 5.2 ($n = 5$)] and in animals collected in the field [89.6 ± 15.9 ($n = 4$)] at the same time that the experiment ended. These results suggest that reproduction draws heavily on lipid reserves and cannot be completely compensated by feeding. Apparently, once the ovaries start to mature, food energy allocation shifts to egg production rather

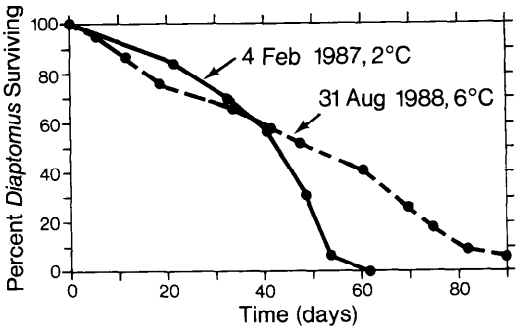

Fig. 5. Survivorship of starved winter and summer *Diaptomus sicilis* females at ambient Lake Michigan temperatures. A χ^2 goodness-of-fit indicated that the two curves were highly significantly different ($P < 0.001$, 4 df).

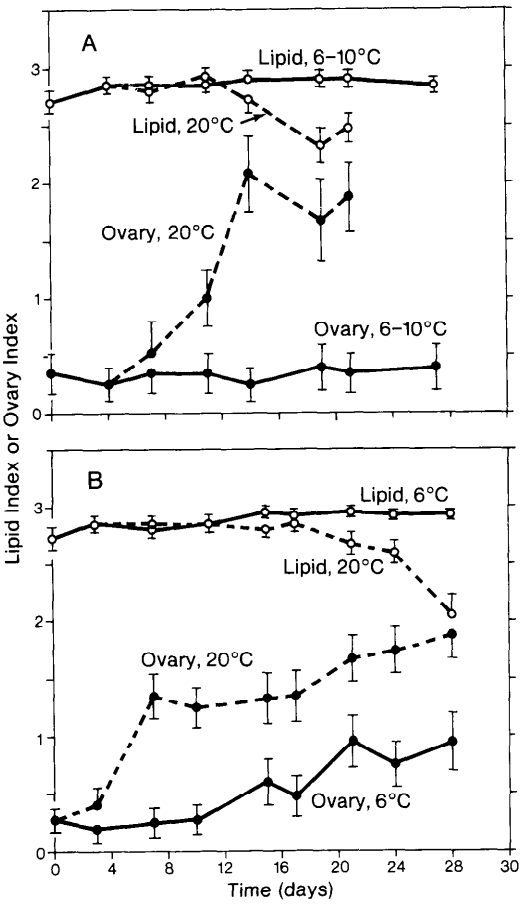

Fig. 6. Lipid and ovary indices for well-fed *Diaptomus sicilis* females kept at 6° and 20°C for experiments starting on 19 September 1986 (A) and 28 September 1987 (B). In experiment A, 63% of *D. sicilis* survived the duration of the experiment at 6°C; mortality was not recorded for 20°C. In experiment B, 67% survived at 6°C and 27% at 20°C.

than lipid storage, and the lipid stores are depleted. Perhaps further deposition did not occur in the 6°C animals because they had reached some physiological limit or metabolism had already shifted over for ovary development.

From observations here and elsewhere (Watras 1983; Williamson and Butler 1987), the following picture emerges on lipids and the life-cycle strategy of *D. sicilis* and other diaptomids. As long as food concentration is adequate, *Diaptomus* is able to accumulate lipids, as witnessed by high concentrations observed in our field study and earlier studies of Siefken and Armitage (1968) and Hairston (1979) as well as in the recent study of Arts and Sprules (1987). The early studies may have been compromised by artifacts associated with measuring lipid concentration as the dry-weight change following extraction of lipid, but the pond-living *Diaptomus* spp. in Siefken and Armitage's study yielded seasonally oscillating lipid concentrations on the same order observed in our study. We have seen that *Diaptomus* can survive for a long time at low temperatures without food, presumably because of its lipid reserves and low metabolic rate at these low temperatures. Egg production is limited by both food and temperature in *Diaptomus* (Watras 1983; Williamson and Butler 1987). Our study showed that TG reserves are gradually drawn down to very low levels during the reproductive period. Thus, these reserves may serve an important function to augment reproductive output as they do in certain marine copepods (Gatten et al. 1980; Ohman 1987). Apparently this reduction of energy reserves occurs even at high food concentration (Fig. 6). As noted above food energy may be shunted into eggs rather than first going into lipid storage before egg production. Further experimental work is necessary to evaluate these hypotheses.

Previous work (Williamson and Butler 1987) proposed that the *f* index may be responsive to food limitation but independent of temperature. Our data indicate that this

Table 2. Triacylglycerol (TG) reserves and survival (from Fig. 5) of starved *Diaptomus sicilis* females during reproductive (February) and nonreproductive (August) seasons. Errors quoted are ±1 SE with sample sizes indicated in parentheses.

	Temp. (°C)	Ovary index	TG reserves		Time (d) to	
			Concn ($\mu g\ \mu l^{-1}$)	Predicted duration* (d)	50% dead	75% dead
4 Feb 87	2	1.48±0.27(25)	32.2±1.2(4)	13†	43	50
31 Aug 88	6	0±0(25)	148.7±14.8(5)	44	50	71

* Calculated from O_2 respiration predictions from temperature and *Diaptomus* length (Comita 1968); an O_2 consumption/lipid conversion factor of 2.03 liters g^{-1} was used (Prosser and Brown 1962).
† The calculation for 2°C is an extrapolation because respiration was not measured below 5°C.

response may not occur at certain temperature ranges for some species of *Diaptomus*. We observed high values of the *f* index simultaneously with high lipid concentrations in field populations and temperature limitation of ovarian development in the laboratory. Time series of both lipid and *f* indices would be useful for distinguishing between periods of food and temperature limitation. For example, our results suggest that if both lipid and *f* indices are high then temperature is the limiting factor. Further generalizations require more experimentation.

It is well known that temperature regulates generation time, egg production rate, and oviducal cycle period in *Diaptomus* and other zooplankton (e.g. McLaren 1978; Watras 1983; Williamson and Butler 1987). We are not aware, however, of any work that relates temperature to duration between molting into the adult stage and ovary maturation. Our results suggest that low temperature limits this maturation. A prereproductive period equivalent to time between clutches may exist (Corkett and McLaren 1978). Both interclutch duration and preadult stage (intermolt) durations varied with temperature; ovary maturation rate is probably slow at low temperatures. When reproduction of *D. sicilis* finally did occur in nature, it was when water temperature was near or at its lowest point in winter or spring. That temperature was at its lowest point when reproduction was at its maximum does not necessarily contradict the temperature-limitation hypothesis. The temperature that *D. sicilis* experiences throughout the year is low (~1°–7°C). We suggest that ovarian development is slow at any of these temperatures, and it turns out that the integrated effect of temperature is visible ovary development occurring in late winter and early spring. Further work is necessary to examine the effects of both food and temperature limitation on ovary maturation and reproductive output.

By residing in the hypolimnion, *D. sicilis* may slow onset of reproduction relative to warmer surface waters. It is probable, however, that total reproductive output is increased because of a more favorable balance between feeding and respiration in the hypolimnion. In any case, the life-cycle strategy of *D. sicilis* works; it is one of the dominant diaptomids in the Great Lakes and is found in the hypolimnia of these lakes. It is both the deepest dwelling and largest diaptomid in the Great Lakes. By dwelling in the hypolimnion, *D. silicis* avoids mortality from visually preying young-of-year and juvenile pelagic fishes that structure the size of the zooplankton in the epilimnion of Lake Michigan through their selective predation of large zooplankton.

We must also consider that *D. sicilis* may remain in deep water to retard ovarian development so that eggs are released in winter and spring when food conditions are probably better for the survival of nauplii and copepodites. We cannot unravel this balance among food limitation, temperature limitation, and predation avoidance until we understand the population and lipid dynamics of the early life stages. Studying the lipid dynamics of these early stages may provide clues to this problem. Visual measurement of lipid stores (Arts and Evans 1991) in these stages would probably be the method of choice because these stages are

small and would contain insufficient lipids for practical chemical or gravimetric measurement.

Henry A. Vanderploeg
Wayne S. Gardner
Christopher C. Parrish[1]
James R. Liebig
Joann F. Cavaletto

Great Lakes Environmental Research Laboratory, NOAA
2205 Commonwealth Blvd.
Ann Arbor, Michigan 48105

References

ARTS, M. T., AND M. S. EVANS. 1991. Measurement of triacylglycerol energy reserves in calanoid copepods. Limnol. Oceanogr. **36:** 289–298.

———, AND G. W. SPRULES. 1987. Energy reserves of three zooplankton species from two lakes with different metal concentrations. Can. J. Fish. Aquat. Sci. **44:** 458–466.

BAMSTEDT, U. 1986. Chemical composition and energy content, p. 1–58. *In* E. D. S. Corner and S. C. M. O'Hara [eds.], The biological chemistry of marine copepods. Clarendon.

BEVINGTON, P. R. 1969. Data reduction and error analysis for the physical sciences. McGraw-Hill.

BOWERS, J. A. 1980. Feeding habits of *Diaptomus ashlandi* and *Diaptomus sicilis* in Lake Michigan. Int. Rev. Gesamten Hydrobiol. **65:** 259–267.

CAVALETTO, J. F., H. A. VANDERPLOEG, AND W. S. GARDNER. 1989. Wax esters in two species of freshwater zooplankton. Limnol. Oceanogr. **34:** 785–789.

COMITA, G. W. 1968. Oxygen consumption in *Diaptomus*. Limnol. Oceanogr. **13:** 51–57.

CORKETT, C. J., AND I. A. MCCLAREN. 1978. The biology of *Pseudocalanus*. Adv. Mar. Biol. **15:** 1–231.

FOLCH, J., M. LEES, AND G. H. SLOANE STANLEY. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. **226:** 497–509.

GARDNER, W. S., W. A. FREZ, E. A. CICHOCKI, AND C. C. PARRISH. 1985. Micromethod for lipids in aquatic invertebrates. Limnol. Oceanogr. **30:** 1100–1105.

GATTEN, R. R., J. R. SARGENT, T. E. V. FORSBERG, S. C. M. O'HARA, AND E. D. S. CORNER. 1980. On the nutrition and metabolism of zooplankton 14. Utilization of lipid by *Calanus helgolandicus* during maturation and reproduction. J. Mar. Biol. Assoc. U.K. **60:** 391–399.

GUILLARD, R. R., AND C. J. LORENZEN. 1972. Yellow-green algae with chlorophyllide *c*. J. Phycol. **8:** 10–24.

HAIRSTON, N. G., JR. 1979. The adaptive significance of color polymorphism in two species of *Diaptomus* (Copepoda). Limnol. Oceanogr. **24:** 15–37.

MCLAREN, I. A. 1978. Generation lengths of some temperate marine copepods: Estimation, prediction, and implications. J. Fish. Res. Bd. Can. **35:** 1330–1342.

MARSHALL, S. M., AND A. P. ORR. 1952. On the biology of *Calanus finmarchicus*. 7: Factors affecting egg production. J. Mar. Biol. Assoc. U.K. **30:** 527–547.

OHMAN, M. D. 1987. Energy sources for recruitment of the subantarctic copepod *Neocalanus tonsus*. Limnol. Oceanogr. **32:** 1317–1330.

PARRISH, C. C. 1987. Separation of aquatic lipid classes by chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. Can. J. Fish. Aquat. Sci. **44:** 722–731.

PROSSER, C. L., AND F. A. BROWN. 1962. Comparative animal physiology. Saunders.

SIEFKEN, M., AND K. B. ARMITAGE. 1968. Seasonal variation in metabolism and organic nutrients in three *Diaptomus* (Crustacea: Copepoda). Comp. Biochem. Physiol. **24:** 591–609.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis, 2nd ed. Bull. Fish. Res. Bd. Can. 167.

TESSIER, A. J., AND C. E. GOULDEN. 1982. Estimating food limitation in cladoceran populations. Limnol. Oceanogr. **27:** 707–717.

———, L. L. HENRY, AND C. E. GOULDEN. 1983. Starvation in *Daphnia*: Energy reserves and reproductive allocation. Limnol. Oceanogr. **28:** 667–676.

TORKE, B. G. 1975. The population dynamics and life histories of crustacean zooplankton at a deep-water station in Lake Michigan. Ph.D. thesis, Univ. Wisconsin–Madison. 91 p.

VANDERPLOEG, H. A. 1990. Feeding mechanisms and particle selection in suspension-feeding zooplankton, p. 183–212. *In* R. S. Wotton [ed.], The biology of particles in aquatic systems. CRC.

———, S. J. BOLSENGA, G. L. FAHNENSTIEL, J. R. LIEBIG, AND W. S. GARDNER. In press. Plankton ecology in an ice-covered bay of Lake Michigan: Utilization of a winter bloom by reproducing copepods. Hydrobiologia.

———, D. SCAVIA, AND J. R. LIEBIG. 1984. Feeding rate of *Diaptomus sicilis* and its relation to selectivity and effective food concentration in algal mixtures and in Lake Michigan. J. Plankton Res. **6:** 919–941.

WATRAS, C. J. 1983. Reproductive cycles in diaptomid copepods: Effects of temperature, photocycle, and species on reproductive potential. Can. J. Fish. Aquat. Sci. **40:** 1607–1613.

WILLIAMSON, C. E., AND N. M. BUTLER. 1987. Temperature, food and mate limitation of copepod reproductive rates: Separating the effects of multiple hypotheses. J. Plankton Res. **9:** 821–836.

Submitted: 5 October 1990
Accepted: 14 March 1991
Revised: 14 November 1991

[1] Current address: Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, P.O. Box 1000, Halifax B3J 2X4.